REMARKS

Claims 1, 2, 6 and 7 are rejected under 35 U.S.C. §102(b) as being anticipated by Nayak et al. (Infection and Immunity 66:3744-3751, 1998). Specifically, the Office alleges that Nayak teaches an attenuated *Salmonella typhimurium* that makes use of a balanced-lethal host-vector system that employs an *asd* deletion in the host and an Asd+ vector to complement the defective host *asd*. The Office asserts that the burden is on Applicant to show a novel or non-obvious difference between the claimed compositions and those of the prior art.

First, note the limitation in currently pending claim 1: "wherein the complementing gene can recombine to replace the non-functional chromosomal essential gene..." Applicant, in a previous response, asserted that the methods used in the Nayak reference resulted in a mutation of the native chromosomal essential gene whereby the flanking sequences are deleted, and therefore the complementing gene harbored by the extrachromosomal vector *can not* recombine to replace the non-functional chromosomal essential gene. The same statement is put forth in the specification of the instant application at page 7, lines 9-24 and also in Example 1 at page 40 beginning at line 16. The Office contends that evidence is required in support of this statement. While Applicant points out that such evidence is not required, because as stated in In re Brana (34 USPQ2d 1436, 1995) "the PTO has the initial burden of challenging a presumptively correct assertion ... in the disclosure." However, in an effort to expedite the examination of this application Applicant offers the following evidence.

As discussed in Example 1 of the instant application, Example 6 of U.S. Patent No. 5,672,345 describes a means of generating Δ*asd* mutations by excision of a Tn*10* linked closely to the *asd* gene. As described in the '345 patent, the mutation is due to Tn*10* insertion into a DNA sequence adjacent to the *asd* gene, and then the Δ*asdA1* mutation arose by fusaric acid resistance selected deletion of the Tn*10* and adjacent gene sequences that included the *asd* gene. As a result, DNA sequences flanking the *asd* gene were deleted also, making it impossible for a wild-type *asd* gene, such as is carried on plasmid pYA3148 or pYA3193, to recombine to functionally replace the deleted *asd* mutation. (It was because of this description that the Examiner, during prosecution of the '345 patent, required that the claims of that patent stipulate the

inability of the vector *asd* gene to recombine to replace the *asd* chromosomal mutation.) Nayak et al. used in their experiments *S. typhimurium* χ4550, which possesses the same Δ*asdA1* (*zhf-4::Tn10*) mutation which was isolated and defined in the '345 patent. (See *materials and methods* section of the Nayak paper, where χ4550 is mentioned, citing a reference co-authored by the instant inventor). Thus, the Nayak et al. reference fails to teach the limitation "wherein the complementing gene can recombine..."

As described in the Examples of the instant application, newer techniques of generating defined deletion mutations by allele replacement enable deletions of just the structural *asd* gene. Thus, when Asd+ vectors with the entire *asd* gene and its flanking 5' promoter and 3' termination sequences are used to complement the *asd* chromosomal mutation it is at least possible that the plasmid-born *asd* gene can recombine to replace the chromosomal mutation, compromising the functional utility of the system. The studies described in the instant application demonstrate that such a system does in fact retain utility, because although recombinational replacement of the chromosomal *asd* mutation is possible, it occurs at such a low frequency as to not compromise the utility of the claimed functional balanced-lethal host-vector system. Thus, this feature (retention of functional utility despite the ability to recombine) constitutes a novel and non-obvious difference between the claimed composition and the composition of the prior art. Applicant has thus met its burden of proof, obviating the rejection.

Claims 1-7 and 12-20 are rejected under 35 U.S.C. §102(b) as being anticipated by Curtiss III et al., (U.S. Patent No. 6,029,961). That patent is also cited as teaching a balanced-lethal host-vector system.

Applicant respectfully points out that because the issue date of the '961 patent (February 15, 2000) is less than a year before the filing date of the instant application (October 11, 2000), that patent is not available as prior art under 35 U.S.C. §102(b). This rejection is therefore improper, and must be withdrawn.

Claims 1, 2, 8-13, 16 and 21-22 are rejected under 35 U.S.C. §102(e) as being anticipated by Portnoy et al. (U.S. Patent No. 6,004,815). The Office cites that patent as teaching an *E. coli* deficient in the production of DAP and a recombinant complementing gene on a vector, citing column 16 of that patent.

Applicant respectfully points out that the Portnoy reference does not teach a vector with a recombinant complementing gene. Also, note that the bacteria mentioned in Table 1 and described in claims 1-6 do not possess a non-functional native chromosomal essential gene, as required by the instant claims. The instant claims comprise a non-functional native chromosomal essential gene. As defined in the specification of the instant application, an "essential" gene is one that encodes a function that is required for cell viability. (See page 14, lines 30-35). The bacteria shown in Table 1 and described in other parts of the Portnoy patent, specifically the Examples, are merely attenuated and do not possess a non-functional native chromosomal essential gene, as the attenuated bacteria remain viable. The Office then points specifically to column 16, where *E. coli* deficient in the production of DAP are mentioned, although the means by which those bacteria are DAP-minus is not disclosed. However, even assuming for the sake of argument that that phenotype is caused by a non-functional native chromosomal essential gene, the reference fails to teach a recombinant complementing gene on an extrachromosomal vector, also required by the instant claims. As further discussed in the Portnoy patent at column 16, lines 45-52, those DAP-minus E. coli harbor plasmid pWR100, and "...have the ability to invade cultured cells and enter the cytosol..., yet following brief replication, spontaneously lyse in the cytosol..." The skilled artisan would understand that the spontaneous lysis of those E. coli is due to the fact that DAP is not produced (see column 16, lines 42-45). A plasmid carrying a recombinant complementing gene would provide DAP production such that the cell would not spontaneously lyse. On this basis, the skilled artisan can determine that plasmid pWR100 does not carry a recombinant complementing gene. Thus, the Portnoy et al. reference fails to teach each element of the claimed invention, and therefore does not anticipate the instant claims.

All of the stated grounds of rejection have been properly traversed, accommodated, or rendered moot. Applicant therefore respectfully requests that the Examiner reconsider all presently outstanding rejections and that they be withdrawn. It is believed that a full and complete response has been made to the outstanding Office Action, and as such, the present application is in condition for allowance. If the

Examiner believes, for any reason, that personal communication will expedite prosecution of this application, he is invited to telephone the undersigned at the number provided.

Prompt and favorable consideration of this Amendment is respectfully requested.

Respectfully submitted,

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Clean Copy of the Amended Claims

45. (New) An attenuated derivative of a pathogenic microorganism which comprises:

(a) a mutation of a polynucleotide sequence that renders a native chromosomal essential gene non-functional;

(b) a recombinant complementing gene on an extrachromosomal vector, wherein the complementing gene is a functional replacement for said essential gene of (a) and wherein said complementing gene can recombine to replace the essential gene of (a); and

(c) a desired gene on the extrachromosomal vector, wherein the desired gene is a recombinant gene encoding a desired gene product;

wherein the desired gene is stably maintained in a progeny population of the microorganism.

46. (New) An attenuated derivative of a pathogenic microorganism which comprises:

(a) a non-functional native chromosomal essential gene;

(b) a recombinant complementing gene on an extrachromosomal vector, wherein the complementing gene can recombine to replace the non-functional chromosomal essential gene;

(c) a desired gene on the extrachromosomal vector, wherein the desired gene is a recombinant gene encoding a desired gene product; and

(d) an inactivating mutation in a native gene selected from the group consisting of a *pab* gene, a *pur* gene, and *aro* gene, *nadA, pncB, gale, pmi, fur, rpsL, ompR, htrA, hemA, cdt, cya, crp, dam, phoP, phoQ, rfc, poxA, falU, mviA, sodC, recA, ssrA, sirA, inv, hilA, rpoE, flgM, tonB,* and *slyA;*

wherein said complementing gene of (b) is a functional replacement for said essential gene of (a), wherein the desired gene is stably maintained in a progeny population of the microorganism.